AWARD NUMBER: W81XWH-11-1-0218

TITLE: Using 3D Super-Resolution Microscopy to Probe Breast Cancer Stem Cells and Their Microenvironment

PRINCIPAL INVESTIGATOR: Lydia Lee Sohn

CONTRACTING ORGANIZATION: University of California
Berkeley, CA 94720

REPORT DATE: May 2014

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
May 2014

2. REPORT TYPE
Final Report

3. DATES COVERED
15 Feb 2011 – 14 Feb 2014

4. TITLE AND SUBTITLE
Using 3D Super-Resolution Microscopy to Probe Breast Cancer Stem Cells and Their Microenvironment

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0218

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHOR(S)
Lydia Lee Sohn

E-Mail: sohn@me.berkeley.edu

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California
Berkeley, CA 94720

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
We report the final achievements of our project. These achievements include the successful building of a super-resolution microscope that is capable of performing 2D and 3D Stochastic Optical Reconstruction Microscopy (STORM) and Photoactivated Localization Microscopy (PALM), and a quantitative-phase imaging (QPI) system that enables the label-free imaging of cells over a period of days. With STORM imaging, we imaged the spatial organization or clustering of the CCR7 receptor on the surface of MCF-7 breast cancer cells that had been cultured on gelatin substrates. This imaging was the first of its kind for breast cancer. We also small controllable microenvironments, in which we can simultaneously culture small groups of cells and monitor their response to external perturbation. We subsequently examined the effects of microenvironments on breast cancer by creating arrays of polydimethlysiloxane (PDMS) microposts of different stiffness and sizes and seeded them with MCF-7 cells. We manipulated our micropost microenvironments to study the effects of microenvironment on cell lineage, namely the basal-luminal differentiation of MCF-7s. Finally, with QPI, we investigated the real-time response of breast-cancer cells to different microenvironmental cues. We thus have begun to learn how protein density, growth rate, morphology, motility, and phenotype of these cells change and evolve in response to their microenvironment.

15. SUBJECT TERMS
breast cancer, MCF-7, basal, luminal, STORM, PALM, quantitative phase imaging, differentiation, growth rate, PDMS stiffness

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	17	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Introduction

In order to increase our understanding of cancer stem cells and their role in cancer progression and metastasis, our overarching goal had been to study *how the cellular microenvironment maintains and controls this cellular phenotype*. To realize this goal, we had proposed to use 3D super-resolution microscopy to visualize how individual breast CaSCs and tumor cells interact with each other and their microenvironment. In Year 1 of this project, we completed the construction of our super-resolution microscope, which had the capabilities to perform both 2D and 3D multi-color Stochastic Optical Reconstruction Microscopy (STORM) and Photoactivated Localization Microscopy (PALM). We demonstrated our ability to image specific receptors on the surface of MCF-7 breast ductal carcinoma cells. In Year 2, we focused on fabricating small controllable microenvironments, in which we can simultaneously culture small groups of cells and monitor their response to external perturbation. We subsequently examined the effects of microenvironments on breast cancer by creating arrays of polydimethlysiloxane (PDMS) microposts of different stiffness and sizes and seeded them with MCF-7 cells. We manipulated our micropost microenvironments to study the effects of microenvironment on cell lineage, namely the basal-luminal differentiation of MCF-7s. In Year 3, we re-built our microscope so that we could perform *label-free* quantitative phase imaging (QPI) and image breast-cancer cells over a 7-day culture. With QPI, we investigate the real-time response of breast-cancer cells to different microenvironmental cues. We thus are able to learn how protein density, growth rate, morphology, motility, and phenotype of these cells change and evolve in response to their microenvironment**.**

Keywords: breast cancer, MCF-7, basal, luminal, STORM, PALM, quantitative phase imaging, differentiation, growth rate, PDMS stiffness

Accomplishments

<u>*Year 1: Super-resolution Imaging of Specific Receptors of Breast-Cancer Cells*</u>. In Year 1, we completed building our super-resolution microscope. This microscope has the capability of performing both two-dimensional (2D) and three-dimensional (3D) Stochastic Optical Reconstruction Microscopy (STORM) and Photoactivated Localization Microscopy (PALM). We focused on the marker CCR7, whose high expression has been correlated with poor survival and whose expression may be regulated by the tumor microenvironment[2]. MCF7 cells were cultured in DMEM with 10% FBS, 0.1 mM MEM non-essential amino acids, and 1 mM sodium pyruvate at 37 °C and 5% CO2. Cleaned coverslips were coated with gelatin attachment factor from Invitrogen and incubated at room temperature for 2 hours or 37C for 30-45 min, and washed with media. The MCF7 cells were dissociated from their culture plate using enzyme-free dissociation buffer from Invitrogen, and transferred to culture wells with the cover sips sitting in the bottom. The cells were subsequently

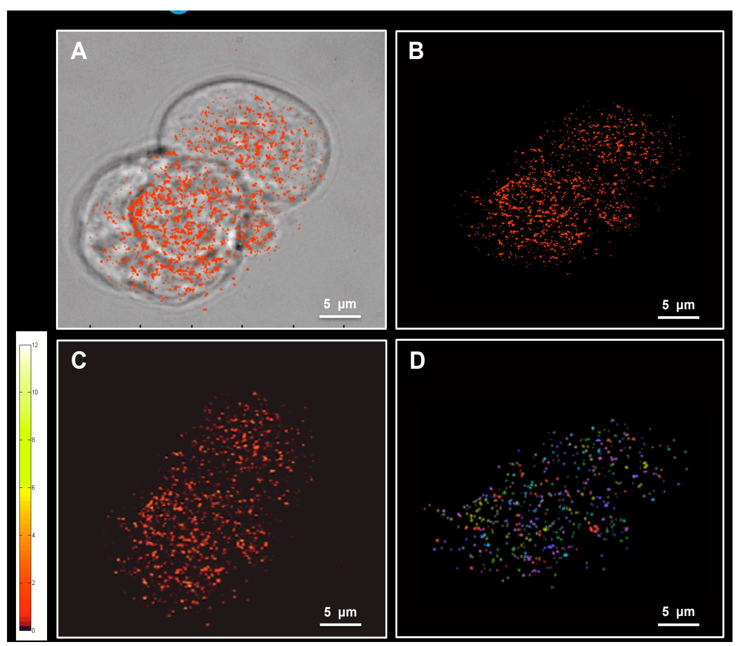

Fig. 1: 2D STORM image of the CCR7 receptor on the surface of MCF-7 breast cancer cells cultured on a gelatin substrate. (A) Bright-field image of two cells with 2D STORM image overlaid on top. Each red "dot" corresponds to an individual CCR7 receptor. Clustering of the receptor is apparent. (B) 2D STORM image of the two cells in (A). (C) Density of localizations or receptors on the surface of the cells. Red corresponds to low density, yellow to high density. (D) Individual clusters of CCR7 receptors on the cells' surface.

cultured overnight before they were fixed and stained with Alexa Fluor 568- (the reporter dye) and Alexa Fluor 405- (the activator dye) conjugated anti-CD197 antibody to thus tag the CCR7 marker on the cell surface. Fig. 1A is bright-field image of two MCF-7 cells overlaid with the 2D STORM image of the CCR7 marker on the surface of these cells. Fig. 1B is solely the 2D STORM image—it is the first super-resolution image of a breast cancer cell to our knowledge. Since clustering or spatial organization of surface markers can influence signaling, we have performed an initial analysis of our 2D STORM images. We have performed a preliminary analysis of our images. Utilizing a Tree-Cluster algorithm that estimates the number of clusters followed by a Gaussian Mixture model that then fits the data based on the estimated number of clusters, we were able to identify each cluster of CCR7 on a cell surface. Fig. 1C shows the density of localizations (i.e. the density of markers in region, with red to yellow corresponding to low to high density) and Fig. 1D identifies each cluster. Fig. 2 shows the 2D STORM images and analyzed results of three different experiments.

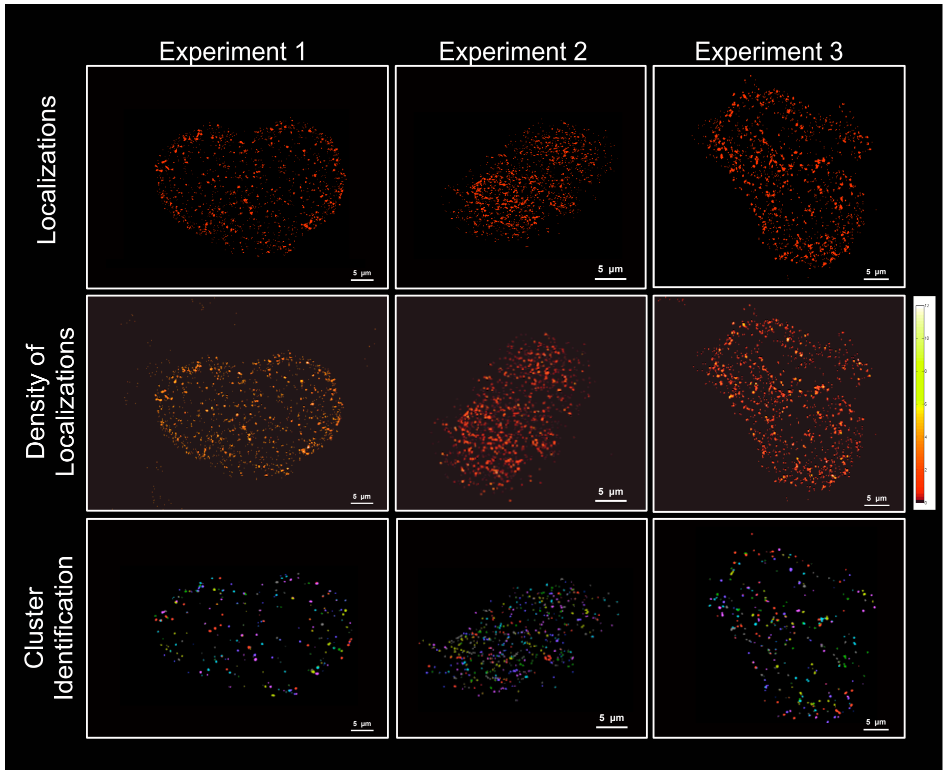

Fig. 2: 2D STORM imaging of the CCR7 receptor on the surface of MCF-7 breast cancer cells cultured on gelatin substrates.

Year 2: *Fabricating Small Controllable Microenvironments for Simultaneously Culturing and Monitoring*. Our initial plan to create the small controllable microenvironments had been to use an appropriate composition of extracellular proteins and proteoglycans (e.g. collagen gels, matrigel, hyaluronic acid gels, and polyacrylamide gels functionalized with various ECM proteins) in a microfluidic reservoir. We would alter dynamically the mechanical properties of this microfluidic culture environment either through the application of stress or strain via mechanical actuators. Given the complexity of the system and the number of parameters involved, we simplified our system greatly by creating tunable micropost arrays to study breast cancer cell microenvironments. These arrays are rapidly and easily fabricated, due to an uncomplicated geometry and simple material selection (polydimethylsiloxane – PDMS). Critically, simple fluid flow profiles can be used, with gradient profiles created using the easily altered aspect ratio and stiffness of the microposts. Advantages of our tunable micropost arrays over traditional two-dimensional tissue-culture polystyrene (TCPS) include improvements in microenvironment accuracy and throughput through control of material properties e.g. stiffness and array size/density.

The fabrication process of our tunable micropost arrays relies on standard soft lithographic techniques. Using AutoCAD, we create a mask and use soft lithography to create PDMS (Sylgard 184) microposts with a given aspect ratio and stiffness (typically 2500-5000Pa for breast tumor conditions[2]). These micropost arrays are highly tunable because altering the stiffness and array size/density is merely dependent on the ratio of PDMS to curing agent and the dimensions of the array, respectively. We chose to create tunable micropost arrays in order to study how the variation of the microenvironment properties using aspect ratio and stiffness affects breast cancer cell differentiation and lineage (basal vs. luminal).

PDMS microposts were created by first constructing silicon master molds using Shipley 1813 positive photoresist. By varying the spin-speed, we are able to control the height of the resulting microposts. PDMS was poured over the master and cured at 80^{o}C for 2 hours. Atomic Force Microscopy (AFM) was used to characterize the height and shape of these microposts (Fig. 3).

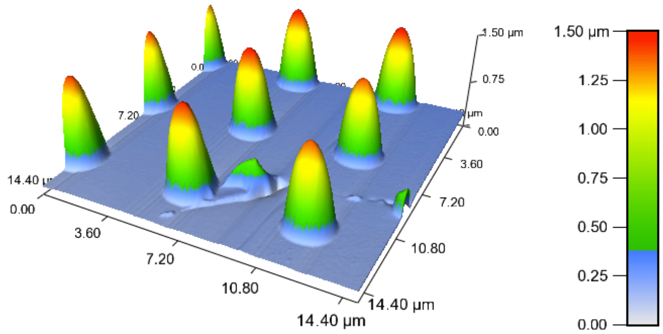

Fig. 3*: AFM was used to characterize micropost size and shape after soft lithography. Based on the above array pattern, we demonstrate an easily tunable micropost array using soft lithographic tools. AFM images were taken using a Veeco Si_3N_4 cantilever, with imaging done in AC mode in air. The sample tested contained 3µm diameter PDMS microposts with a 6µm lattice constant.*

MCF-7 breast ductal carcinoma cells were successfully cultured on our PDMS micropost arrays for over a week in DMEM (Gibco) + 10% fetal bovine serum (Gibco) + 1% penicillin/streptomycin (Invitrogen) (Fig. 4). The devices are optically transparent so further analysis such as bright field imaging and immunostaining are facile.

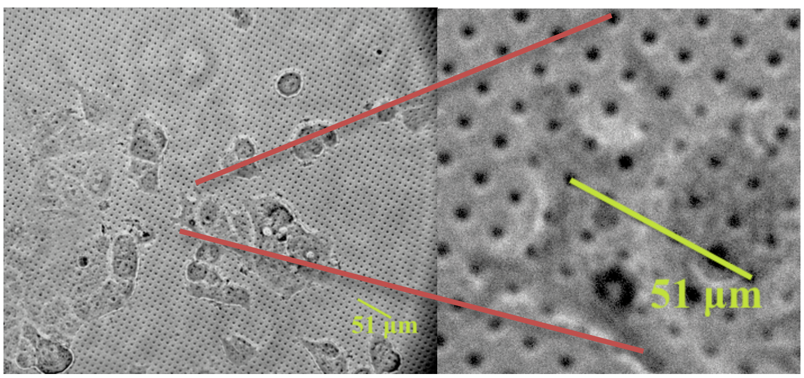

Fig. 4*: Micropost array with MCF-7 breast ductal carcinoma cells plated. Image was taken following one day of culturing on the micropost array. Generous cell spreading and morphology indicate normal cell growth and biocompatibility of our device.*

Based on these initial results, we have created a high-throughput analysis system (Fig. 5) that would allow us to investigate the effect of stiffness range on phenotype (given that tumors are heterogeneous in terms of stiffness). We are currently focusing on the MCF-7 a-CD271 phenotype since it has been recently reported to be a candidate breast CaSCs[3].

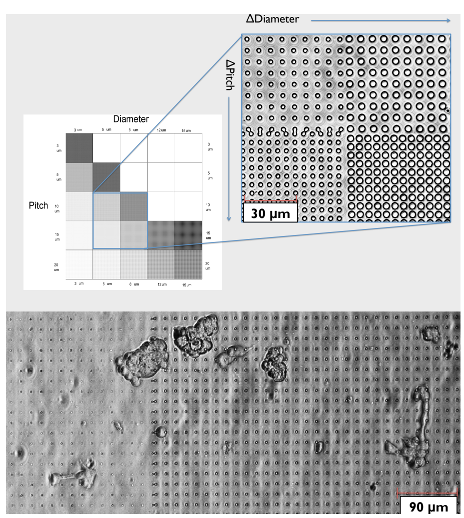

Fig. 5: *(**Top**) In order to test multiple stiffness conditions on a single, high-throughput chip, we have developed a “gradient” micropost array. (**Bottom**) Cell spreading and morphological changes are observed between areas of differing apparent modulus following cell seeding.*

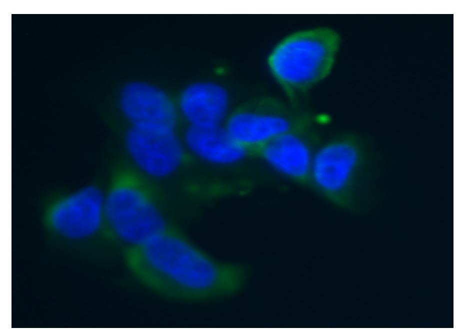

Fig. 6: *MCF-7 breast ductal carcinoma cells stained with an α-CD271 antibody (Abcam p75 NGF). The α-CD271 marker has recently been reported as a stem-like marker for pre-tumorigenic cells.*

Through immunostaining, we have identified that phenotype with our MCF-7 cell line (Fig. 6).

Year 3: Quantitative-Phase Imaging. Conventional label-free microscopy, such as phase contrast (PC) and differential interference contrast (DIC), are widely used to provide morphometric information without the use of any exogenous contrast agents. The "contrast" in these techniques is the actual phase shift induced by the difference in the optical path length through a cell as compared to its surroundings. Although the optical phase shift provides information regarding protein-density distribution and total dry mass[4-6], this is lost in PC and DIC due to their qualitative nature. In contrast, QPI measures these *changes in optical path length with nanometer sensitivity*. With this exquisite sensitivity, one can measure changes in dry mass (non-aqueous content) on the order of femtograms over a period of time and obtain a broad view of metabolic activity[4]. Simultaneously, one can also obtain quantitative information on inter- and intra- cellular mass transport[5], spatial organization of cells and tissues[6, 7], and cellular morphology and motility[8]. *QPI is label-free and non-invasive, and thus, it is an ideal technique to measure the fundamental properties of a naturally evolving biological system in an unprecedented manner.*

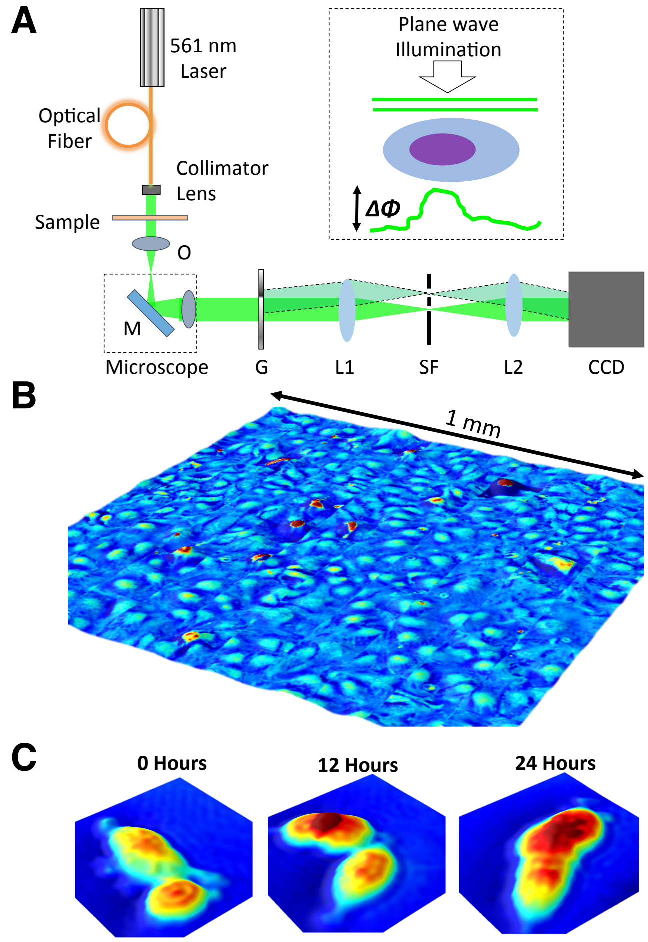

Fig. 7: Diffraction-Phase Microscopy. A) *Schematic of DPM setup. A 561 nm Laser is used to provide plane wave illumination via an optical fiber and collimator lens. A standard microscope objective (O) and inverted microscope is used to project a magnified image of the sample onto a diffraction grating (G). The grating, lens L1, spatial filter (SF) and lens L2 and CCD form a compact common path interferometer. Inset illustrates the phase shift ΔΦ induced by the specimen on the incident plane wave, which is quantified by DPM. ΔΦ is proportional to the product of the refractive index and the thickness of the sample.* ***B)*** *A 1 x 1 mm^2 area mosaic scan of an MCF-7 culture. The colors indicate the phase shift at each point.* ***C)*** *DPM images of a pair of MCF-7 cells as they grow and divide over 24 hours.*

Modifying the STORM/PALM microscope that we created in Year 1, we constructed a fully automated diffraction-phase microscope (DPM) system, equipped with a stage-top incubation system. DPM is a single-shot, wide-field, common-path QPI modality (Fig. 7A). We have used this system to image MCF-7 breast-cancer cells (Fig. 7B and C). An automated stage allows for scanning a large area in a mosaic fashion, thereby providing a large field of view while maintaining sub-cellular resolution. As well, a stage-top incubator enables measurements over biologically relevant timescales. Thus, *we have the capability to measure the behavior of a cell culture over temporal scales ranging from milliseconds to days and spatial scales ranging from sub-micron to centimeters.*

We are currently using this system to investigate the real-time response of breast-cancer cells and neuronal progenitor cells to different microenvironmental cues. Our goal is to learn how protein density, growth rate, morphology, motility, and phenotype of these cells change and evolve in response to their microenvironment**.** Fig. 8 shows the preliminary results of our investigation of how 5 combinatorial protein environments known to play a role in the epithelial-mesenchymal transition (EMT) of breast cancer influence the proliferation dynamics of MCF-7 breast-cancer cells. The protein environments were printed on a glass substrate using a technique developed by our collaborator, Dr. Mark LaBarge at Lawrence Berkeley National Laboratory (LBNL), and live-cell imaging was performed over a 45-hour period.

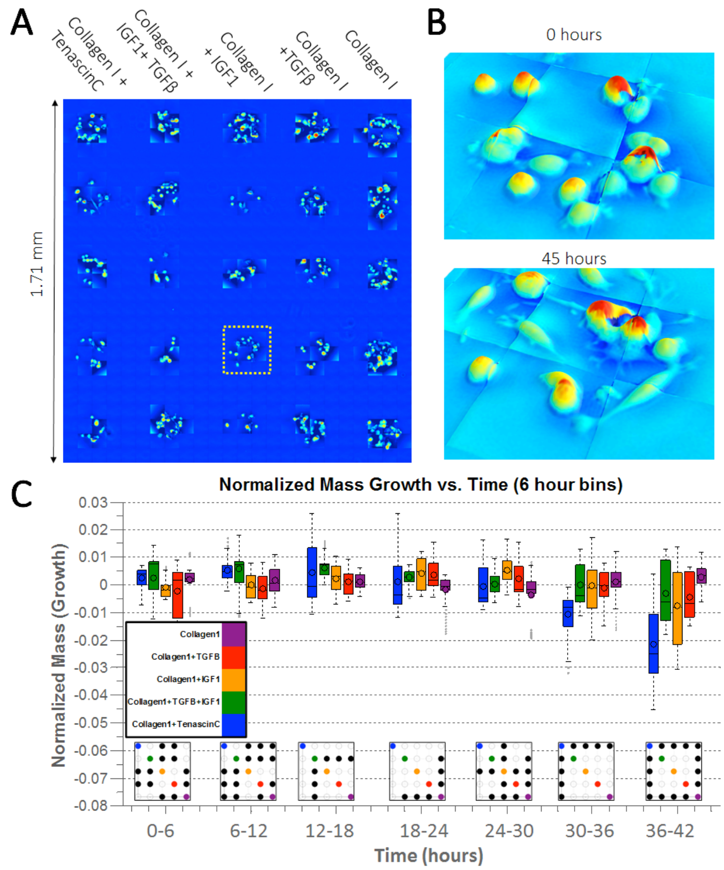

__Fig. 8: Microenvironment array (MEArray) measured using diffraction phase microscopy (DPM). A__) The MEArray shown contains 5 unique environments and 5 replicates of each. The proteins that were patterned on the surface are known to play a role in EMT in breast cancer. MCF-7 breast cancer cells were cultured on the array and scanned using DPM every 30 minutes for 72 hours. The total scanned area was 1.71 x 1.60 mm^2. Scanning involved tiling together 600 images in order to maintain sub-cellular resolution. __B)__ Magnified view of the spot outlined in yellow in __(A)__ at 0 hours and 45 hours. The colors indicate the dry-mass density at each point. The total dry mass vs. time for each spot in the array is calculated by integrating the dry-mass density map over the area of each spot at every time point. __C)__ Normalized mass growth vs. time for all conditions in 6-hour time bins over 45 hours. Insets at bottom indicate which groups have statistically significant differences in growth rate in each time period. Each row in the insets corresponds to the spot type being tested, as indicated by the colored dot, and each column corresponds to the spot type against which it is being compared. Statistically significant (p>0.95) differences are indicated by the black dots. During the first 24 hours, there is a statistically significant higher growth in the Collagen + TGFβ+IGF condition as compared to all other spots. By 45 hours, the growth rate of Collagen1+TenascinC group is significantly lower than all other, indicating that there is higher survivability and proliferation in all other groups vs. the Collagen1+TenascinC population.

Impact

The work described here was high risk, and we made significant headway into developing a set of technologies that will help elucidate the mechanisms for, and biology of, breast cancer. In particular, quantitative phase imaging allows us to go beyond current end-point analyses (e.g. immunocytochemistry, Western Blotting, PCR in which cells are fixed and/or lysed) by performing real-time kinetic measurements of fundamental cell behavior. Performing such measurements, without affecting cell viability and multipotency, enables previously intractable studies regarding the understanding of how various environmental stimuli affect individual cells during the differentiation and growth process. With our QPI studies, we anticipate that we will be able to contribute greatly to the understanding of what role the cellular microenviroment plays in cancer progression.

Changes/Problems
None.

Products
Conference Abstracts and Proceedings
1. Evan Lyall, Matthew Chapman, and L. L. Sohn, "Characterizing Spatial Organization of Cell Surface Receptors in Human Breast Cancer with STORM," Oral Presentation, American Physical Society Annual March Meeting, Boston, MA, March 2012.
2. Matthew Chapman, Evan Lyall, and L. L. Sohn, "Characterizing Spatial Organization of Cell-Surface Receptors in Human Breast Cancer with STORM," Oral Presentation, American Chemical Society Annual Meeting, San Diego, March 2012
3. Anand Kesavaraju, Bo Qing, Eric Jabart, Marc LaBarge, and Lydia L. Sohn, "Micropost microenvironments for studying luminal-basal lineage commitment of breast-cancer cells", Oral Presentation, American Physical Society Annual March Meeting, Baltimore, MD, March 2013.
4. Anand Kesavaraju, Bo Qing, Eric Jabart, and Lydia L. Sohn, "Tunable Micropost Arrays for Studying Breast Cancer Microenvironments", Poster presentation, 7th International Conference on Microtechnologies in Medicine and Biology, Marina Del Ray, CA, April 10-12, 2013.
5. Mustafa Mir, Tiina Jokela, Marc LaBarge, and Lydia L. Sohn, "Quantifying the effects of micro-environments on breast cancer cell proliferation using quantitative phase imaging", submitted to SPIE Photonics West: Quantitative Phase Imaging, July 2014.

Technologies
1. STORM/PALM microscope capable of nm resolution: microscope is available to campus community
2. Quantitative-Phase Imaging microscope capable of label-free imaging: microscope is available to campus community
3. Tunable micropost arrays to study breast-cancer cell microenvironments

Participants
1. Lydia L. Sohn, PI
2. Matthew R. Chapman, Graduate Student Researcher (2010-2012): developed STORM/PALM imaging system
3. Eric Jabart, Graduate Student Researcher (2012-2014): developed and performed experiments on creating tunable micropost arrays to study breast cancer cell microenvironments
4. Mustafa Mir, Ph.D., Postdoctoral Fellow (2013-2014): developed QPI system and performed experiments on MEArrays
5. Evan Lyall, Undergraduate Researcher (2012): assisted in development of STORM/PALM imaging system
6. Bo Qing, Undergraduate Researcher (2012-2013): developed and performed experiments on creating tunable micropost arrays to study breast cancer cell microenvironments
7. Anand Kesavaraju, Undergraduate Researcher (2012-2013): developed and performed experiments on creating tunable micropost arrays to study breast cancer cell microenvironments
8. Marc LaBarge, Ph.D., Collaborator, Lawrence Berkeley National Lab (2014): provided MEArrays and served as consultant on breast cancer and the microenvironment.

Other Collaborating Organizations
Not Applicable

Special Reporting Requirements
None

Appendices

Meeting Abstracts

1. Evan Lyall, Matthew Chapman, and L. L. Sohn, "Characterizing Spatial Organization of Cell Surface Receptors in Human Breast Cancer with STORM," Oral Presentation, American Physical Society Annual March Meeting, Boston, MA, March 2012.

Bulletin of the American Physical Society

APS March Meeting 2012
Volume 57, Number 1

Monday–Friday, February 27–March 2 2012; Boston, Massachusetts

APS Home | APS Meetings | Join APS | Help

Session Y41: Focus Session: Physics of Cancer III -- Imaging
8:00 AM–10:12 AM, Friday, March 2, 2012
Room: 156B

Sponsoring Unit: DBIO
Chair: Robert Austin, Princeton University

Abstract ID: BAPS.2012.MAR.Y41.4

Abstract: Y41.00004 : Characterizing Spatial Organization of Cell Surface Receptors in Human Breast Cancer with STORM
9:24 AM–9:36 AM

Preview Abstract   *MathJax* ***Off*** | *On* ← *Abstract* →

Authors:

Evan Lyall
(Department of Bioengineering, University of California, Berkeley)

Matthew R. Chapman
(Biophysics Graduate Group, University of California, Berkeley)

Lydia L. Sohn
(Department of Mechanical Engineering, University of California, Berkeley)

Regulation and control of complex biological functions are dependent upon spatial organization of biological structures at many different length scales. For instance Eph receptors and their ephrin ligands bind when opposing cells come into contact during development, resulting in spatial organizational changes on the nanometer scale that lead to changes on the macro scale, in a process known as organ morphogenesis. One technique able to probe this important spatial organization at both the nanometer and micrometer length scales, including at cell-cell junctions, is stochastic optical reconstruction microscopy (STORM). STORM is a technique that localizes individual fluorophores based on the centroids of their point spread functions and then reconstructs a composite image to produce super resolved structure. We have applied STORM to study spatial organization of the cell surface of human breast cancer cells, specifically the organization of tyrosine kinase receptors and chemokine receptors. A better characterization of spatial organization of breast cancer cell surface proteins is necessary to fully understand the tumorigenisis pathways in the most common malignancy in United States women.

To cite this abstract, use the following reference: http://meetings.aps.org/link/BAPS.2012.MAR.Y41.4

2. Matthew Chapman, Evan Lyall, and L. L. Sohn, "Characterizing Spatial Organization of Cell-Surface Receptors in Human Breast Cancer with STORM," Oral Presentation, American Chemical Society Annual Meeting, San Diego, March 2012

3. Anand Kesavaraju, Bo Qing, Eric Jabart, Marc LaBarge, and Lydia L. Sohn, “Micropost microenvironments for studying luminal-basal lineage commitment of breast-cancer cells”, Oral Presentation, American Physical Society Annual March Meeting, Baltimore, MD, March 2013.

Bulletin of the American Physical Society

APS March Meeting 2013
Volume 58, Number 1
Monday–Friday, March 18–22, 2013; Baltimore, Maryland

APS Home | APS Meetings | Join APS | Help

Session T45: Focus Session: Physics of Cancer I
8:00 AM–11:00 AM, Thursday, March 21, 2013
Hilton Baltimore Room: Holiday Ballroom 4

Sponsoring Unit: DBIO
Chair: Kimberly Stroka, Johns Hopkins

Abstract ID: BAPS.2013.MAR.T45.6

Abstract: T45.00006 : Micropost microenvironments for studying luminal-basal lineage commitment of breast cancer cells
9:24 AM–9:36 AM

Preview Abstract

MathJax ***On*** | *Off* ← *Abstract* →

Authors:

Anand Kesavaraju
(Department of Bioengineering, Univeristy of California, Berkeley)

Bo Qing
(Department of Bioengineering, Univeristy of California, Berkeley)

Eric Jabart
(Department of Bioengineering, Univeristy of California, Berkeley)

Mark LaBarge
(Lawrence Berkeley National Laboratory)

Lydia Sohn
(Department of Mechanical Engineering, Univeristy of California, Berkeley)

MCF-7 breast cancer cells were plated onto polydimethylsiloxane (PDMS) microposts in order to examine the effects of the microenvironment on cell lineage. Different stiffnesses and sizes of the microposts are postulated to impact cell surface marker expression levels. We will provide preliminary results analyzing CD271 and focal adhesion markers such as vinculin. 3D shear flow will also be applied to the microposts to study how external mechanical stimuli affect cancer cells within their microenvironment.

To cite this abstract, use the following reference: http://meetings.aps.org/link/BAPS.2013.MAR.T45.6

4. Anand Kesavaraju, Bo Qing, Eric Jabart, and Lydia L. Sohn, “Tunable Micropost Arrays for Studying Breast Cancer Microenvironments”, Poster presentation, 7th International Conference on Microtechnologies in Medicine and Biology, Marina Del Ray, CA, April 10-12, 2013.

TUNABLE MICROPOST ARRAYS FOR STUDYING BREAST CANCER MICROENVIRONMENTS

A. Kesavaraju[1], E. Jabart[1], B. Qing[1], L.L. Sohn[2]
[1]University of California, Berkeley Department of Bioengineering, Berkeley, USA
[2]University of California, Berkeley Department of Mechanical Engineering, Berkeley USA

ABSTRACT

Breast cellular microenvironments play a key role in maintaining healthy tissue and disregulation can lead to cancer or promote disease progression. In order to examine the effects of microenvironments on breast cancer, we created arrays of polydimethylsiloxane (PDMS) microposts of different stiffness and sizes and seeded them with MCF-7 breast ductal carcinoma cells.

We manipulated our micropost microenvironments to study the effects of microenvironment on cell lineage, namely the basal-luminal differentiation of MCF-7s. Differentiation of MCF-7s can be monitored optically by morphological analysis, via surface marker expression, PCR, and western blotting. We will provide preliminary results analyzing CD271 (a basal-like cell marker), milk mucin (MM, a luminal-like cell marker), and focal adhesion markers such as vinculin.

KEYWORDS: Microfluidics, microenvironment, microposts, breast cancer, stiffness

INTRODUCTION

Breast cancer and the cellular microenvironment

Breast cellular microenvironments play a key role in maintaining healthy tissue and disregulation of these microenvironments can lead to cancer or promote disease progression. The two primary types of breast cancer cells, basal-like and luminal-like, play different roles in breast cancer and its progression[1]. Although both cell types seemingly arise from luminal-like cells[2], basal-like cells take on a more stem-cell-like phenotype, while luminal cells become more differentiated[3]. Recent work has shown that FACS-purified $CD271^+$ basal-like cells can give rise to both $CD271^+$ and MM^+ (luminal-like), while FACS-purified MM^+ cells gave rise to only MM^+ luminal cells[1]. In this paper we seek to analyze the effects of the cellular microenvironment on the establishment, maintenance, and differentiation of these two cells types in breast cancer progression. In order to study the microenvironment, we created tunable PDMS micropost arrays of different stiffness, shapes, and sizes and seeded them with MCF-7 breast ductal carcinoma cells.

Microfluidics as a tool for studying microenvironments

Microfluidic tools for cell culture are distinguished from more traditional microfluidic applications in chemistry or physics through material, geometry, and fluid flow selections. The near-infinite variety of geometries leads to novel culturing schemes allowing the creation of 3D microenvironments[4]. Recently, microfluidics have been used to study 3D cellular microenvironments through selective partitioning of fluidic channels to create compartmentalized microenvironments[5]. Microfluidic devices able to separate microstructures in gels have also been recently developed[6] and micropillars have been fabricated to act as channel-guides to create specific concentration gradient profiles[7].

Building on these ideas, we have created tunable micropost arrays to study breast cancer cell microenvironments. These arrays are rapidly and easily fabricated, due to an uncomplicated geometry and simple material selection (polydimethylsiloxane – PDMS). Critically, simple fluid flow profiles can be used, with gradient profiles created using the easily altered aspect ratio and stiffness of the microposts. Advantages of our tunable micropost arrays over traditional two-dimensional tissue-culture polystyrene (TCPS) include improvements in microenvironment accuracy and throughput through control of material properties e.g. stiffness and array size/density.

In this paper, we show the development and fabrication of these tunable micropost arrays, along with testing with MCF-7 breast cancer cells to study the generation, maintenance, and differentiation of the basal and luminal subtypes in controlled microenvironments.

METHODS AND RESULTS

The fabrication process of our tunable micropost arrays relies on standard soft lithographic techniques. Using AutoCAD, we create a mask and use soft lithography to create PDMS (Sylgard 184) microposts with a given aspect ratio and stiffness (typically 2500-5000Pa for breast tumor conditions[8]).

These micropost arrays are highly tunable because altering the stiffness and array size/density is merely dependent on the ratio of PDMS to curing agent and the dimensions of the array, respectively. We chose to create tunable micropost arrays in order to study how the variation of the microenvironment properties using aspect ratio and stiffness affects breast cancer cell differentiation and lineage (basal vs. luminal).

PDMS microposts were created by first constructing silicon master molds using Shipley 1813 positive photoresist. By varying the spin-speed, we are able to control the height of the resulting microposts. PDMS was poured over the master and cured at 80^{o}C for 2 hours. Atomic Force Microscopy (AFM) was used to characterize the height and shape of these microposts (Figure 1).

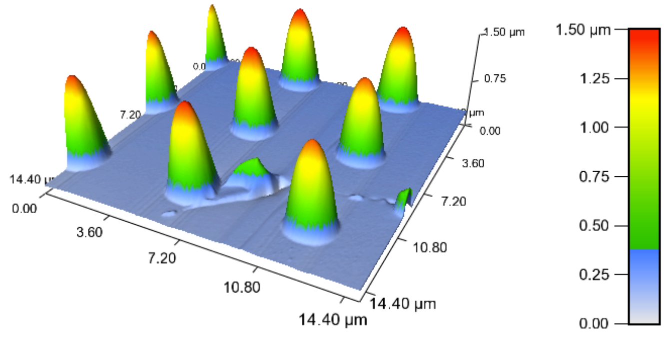

***Figure 1**: AFM was used to characterize micropost size and shape after soft lithography. Based on the above array pattern, we demonstrate an easily tunable micropost array using soft lithographic tools. AFM images were taken using a Veeco Si_3N_4 cantilever, with imaging done in AC mode in air. The sample tested contained 3µm diameter PDMS microposts with a 6µm lattice constant.*

MCF-7 breast ductal carcinoma cells were successfully cultured on our PDMS micropost arrays for over a week in DMEM

(Gibco) + 10% fetal bovine serum (Gibco) + 1% penicillin/streptomycin (Invitrogen) (Figure 2). The devices are optically transparent so further analysis such as bright field imaging and immunostaining are facile.

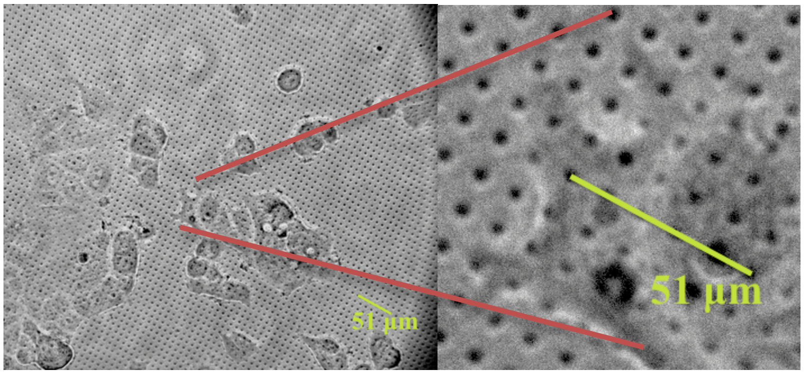

Figure 2*: Micropost array with MCF-7 breast ductal carcinoma cells plated. Image was taken following one day of culturing on the micropost array. Generous cell spreading and morphology indicate normal cell growth and biocompatibility of our device.*

CONCLUSIONS AND FUTURE WORK

Future work will focus on the effects of micropost stiffness on cellular stresses, mechano-sensing protein organization, and the MCF-7 differentiation process.

REFERENCES

[1] Kim J. *et al*. "Tumor initiating but differentiated luminal-like breast cancer cells are highly invasive in the absence of basal-like activity", in *Proc. Nat. Acad. Sciences*. 2012. 109 (16) 6124-6129
[2] Molyneux G, *et al*. "BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells." *Cell Stem Cell* 2010. **7**:403–417.
[3] Shipitsin M, *et al*. "Molecular definition of breast tumor heterogeneity." *Cancer Cell* 2007. **11**:259–273.
[4] Young E.W.K. & Beebe D.J. "Fundamentals of microfluidic cell culture in controlled microenvironments" *Chem. Soc. Rev.*, 2010, 39, 1036-1048.
[5] Zhang C. *et al*. "Towards a human-on-chip: Culturing multiple cell types on a chip with compartmentalized microenvironments". *Lab on a Chip*. 2009. 9, 3185–3192.
[6] Nelson, C.M. & Tien, J. "Microstructured extracellular matrices in tissue engineering and development." *Curr. Op. Biotech.* Volume 17, Issue 5, October 2006, Pages 518–523.
[7] Kim M.S. *et al*. "Microfluidic Micropillar Arrays for 3D Cell Culture." The Open Biotechnology Journal, 2008, 2, 224-228.
[8] Paszek, M. J. *et al*. "Tensional homeostasis and the malignant phenotype." *Cancer Cell* 8, 241–254 (2005).

5. Mustafa Mir, Tiina Jokela, Marc LaBarge, and Lydia L. Sohn, "Quantifying the effects of micro-environments on breast cancer cell proliferation using quantitative phase imaging", submitted to SPIE Photonics West: Quantitative Phase Imaging, July 2014.

Quantifying the effects of micro-environments on breast cancer cell proliferation using quantitative phase imaging

Mustafa Mir[1], Tiina Jokela[2], Mark A. LaBarge[2], Lydia L. Sohn[1]

1) California Institute for Regenerative Medicine, University of California, Berkeley
2) Life Sciences Division, Lawrence Berkeley National Laboratory
Berkeley, California, 94720

Abstract:

Like many other cell functions, metastases and cancer-cell differentiation are directed by complex interactions with the specific microenvironmental niche a cell inhabits. To investigate these interactions, LaBarge et al. (*Stem Cell Rev., 2007*) have developed a parallel microenvironment microarray (MEArray) platform, which allows one to investigate distinct components of the microenvironment (e.g. Collagen, TGF-β, and IGF1) in a combinatorial manner. Currently, measurements on MEArrays have been limited to end-point immunofluorescence measurements or time-lapse measurements using qualitative techniques such as phase-contrast. Here, we demonstrate that we are able to perform label-free quantitative time-lapse studies of cells cultured on MEArrays through Diffraction Phase Microscopy (DPM), a common-path, off-axis, quantitative phase-imaging modality. In particular, we measured the effects of a combinatorial array of microenvironments (Collagen1, TGF-β, IGF1, and TenascinC) that have been known to induce an epithelial-mesenchymal transition (EMT) in MCF-7 breast-cancer cells. We show that we are able to detect significant differences in the proliferation dynamics of cells grown in different environments using DPM. Our findings suggest that quantitative

phase-imaging measurements can be used to identify EMT, a process associated with metastasis, in a label-free manner. Furthermore, such quantitative, multi-parametric, time-lapse measurements can offer a unique insight into the role of the microenvironment in cancer progression.

Summary:
Interactions between cells and their microenvironment play a key role in cancer progression and metastasis. Recently, a combinatorial microenvironment microarray platform has been developed to measure the effects of different microenvironment components in a highly parallelized manner. Here, we demonstrate that time-lapsed quantitative phase imaging of cells cultured on this platform elucidates the effects of the microenvironment on cell proliferation dynamics. We show quantitative-phase imaging results on MCF-7 breast-cancer cells grown on combinatorial microenvironments known to induce epithelial-mesenchymal transitions, a key step in cancer metastasis.

References

1. Wilson, JL, Burchell, J, and Grimshaw, MJ. Endothelins induce CCR7 expression by breast tumor cells via endothelin receptor A and hypoxia-inducible factor-1, Cancer Res. (2006); 11802-7.
2. Kim J. *et al*. “Tumor initiating but differentiated luminal-like breast cancer cells are highly invasive in the absence of basal-like activity”, in *Proc. Nat. Acad. Sciences*. 2012. 109 (16) 6124-6129
3. Molyneux G, *et al*. “BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells.” *Cell Stem Cell* 2010. **7**:403–417.
4. Mir, M. et al. Optical measurement of cycle-dependent cell growth. Proceedings of the National Academy of Sciences of the United States of America 108, 13124-13129 (2011).
5. Wang, R. et al. Dispersion-relation phase spectroscopy of intracellular transport. Optics express 19, 20571-20579 (2011).
6. Ding, H., Wang, Z., Nguyen, F., Boppart, S.A. & Popescu, G. Fourier transform light scattering of inhomogeneous and dynamic structures. Physical review letters 101, 238102 (2008).
7. Hunter, M. et al. Tissue self-affinity and polarized light scattering in the born approximation: a new model for precancer detection. Physical review letters 97, 138102 (2006).
8. Mir, M. et al. Visualizing Escherichia coli sub-cellular structure using sparse deconvolution Spatial Light Interference Tomography. PloS one 7, e39816 (2012).

References

1. Paszek, M. J. *et al*. “Tensional homeostasis and the malignant phenotype.” *Cancer Cell* 8, 241–254 (2005).
2. Kim, J. *et al.* “Tumor initiating but differentiated luminal-like breast cancer cells are highly invasive in the absence of basal-like activity, PNAS doi: 10.1073/pnas.1203203109